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ORIGINAL ARTICLE

Effects of Feeding Rapeseed Meal on the Phosphatase Enzymes of Japanese Quail Liver

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ABSTRACT

A detailed study was undertaken in order to determine the safe dose of mustard seed meal in the diet for Japanese quails which can be used at the production level without having deleterious effects on the growth of this bird. For this purpose, 1500 birds were fed different levels of mustard seed meal in isocaloric and isonitrogenous diets. The results of feeding of these diets for 30 days show that there are no toxic effects on the growth of birds but concentrations of both enzymes (Alkaline phosphatase and Acid phosphatase of liver) decreased in the first 10 days of age, more markedly for alkaline phosphatase. Than enzymes started increasing and after 22 days, their behavior differed again. Biochemical studies were performed on the liver show that at 30 days age the activity of the acid phosphatase decreased but the activity of alkaline phosphatase did not,

Key words: *glucosinolate, Alkaline Phosphatase, Acid Phosphatase, Liver Enzymes.*

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INTRODUCTION

In Pakistan poultry industry has developed much during last two decades. In order to rear chickens for meat purpose, a huge capital is required in term of shelter, feed, vaccine, medication etc. whereas quail farming requires less capital as well as shelter, feed, vaccination and medication. Among all these factors feed amounts to about 70% of total cost of production. So, important factor is to produce most economical poultry production through balanced and cost effective ration. Whereas quail farming in our home land is becoming a popular business, considered to be more profitable, many small farmers are switching over to quail farming [1]. Studies indicated that Ration "C" containing 15% canola meal and 15% soybean meal under local conditions gave better weight gain, feed efficiency and dressing percentage and was the economical. But higher amounts of canola meal were used in diets (20, 25 and 30%) did not showed good results. It is pertinent to mention here that presence of intrinsic antinutrient factors like glucosinolate still found in canola meal in a minute amount may impair the performance, while higher percentage of fiber may also effect when higher levels of canola meal are included in diet [2].

Solid state fermentation (SSF) has been reported to be an effective way to reduce anti-nutritional factors in rapeseed meal, such as glucosinolate while producing a certain amount of rapeseed peptides. Furthermore, these fermented rapeseeds are highly digestible and nutritious, contributing important nutrients including rapeseed peptide, calcium, phosphate, and vitamins B [3]. It is not known whether these rapeseed peptides can improve the immune function of animals. Isothiocyanates can cause goiter and then the swelling of the thyroid may lead to the abnormal secretion of T3 and T4. Recent investigations have shown the use of fermented rapeseed meal can improve pig performance and influence serum biochemical parameters [4, 5]

The average serum alkaline phosphatase (AP) values for the male quails too differed between sexes; the values being higher in the male birds [6] too reported increase in AP values of quails suffering from aflatoxicosis. The detailed review given above clearly demonstrates that rapeseed meals, Brassica seed meals and raw soybeans contain different compounds which are proven inhibitors of growth. The mechanism of action of these compounds may be slightly different, but mainly they inhibit the thyroid hormone synthesis and release within the body. The inhibition of thyroid gland brings changes not only in

the growth of animals, but also changes in the metabolism of different tissues and body organs [7]. Surprisingly, these metabolic changes in body tissues and organ have not been studied in detail, or put in another way, have not been elucidated and documented properly. In addition, a review of the available literature has also shown a lot of lacunae in our knowledge of the changes in growth and metabolism of birds, caused by other plant proteins.

Feeding 20% lower rapeseed meal (a low glucosinolate variety) ration to broiler chicken did not affect the eating quality of the cooked meat. Span variety of rapeseed meal [8] with no amino acid supplementation in a semipurified diet depressed growth in broiler when all the protein was coming from rapeseed meal. The addition of all amino acids known to be limiting (methionine, lysine and arginine) did not completely overcome this growth depression when compared to a soybean meal control diet. When rapeseed meal was included in practical corn soybean type diets, amino acid supplementation has no effect on performance. With small initial investment, one can start a quail farm either in specific location or in his house. Dressed quail meat fetches a reasonable market price ranging from Rs.14 to 25 per bird. Steam roasted are precious item of hotels and restaurants, because quail meat has a definite game bird flavour and reputed to be very tasty tender and delicious with high calorific and low cholesterol value [2].

In view of the above, a study was undertaken in order to evaluate the dietary suitability of mustard seed meal achieved after the extraction of oil from the mustard seeds. This meal was incorporated at three dietary levels, that is, 5, 15 and 25% of the diet. The diets prepared were isonitrogenous and isocloric and were fed to the day old chicks for the first month of their life (30 days) and its effect was seen on the growth of liver of Japanese quail. The aim of study is to develop cheap and economically suitable diet for birds.

MATERIALS AND METHODS

Animal husbandry

A total of 1450 day-old quail chicks were purchased from a commercial hatchery. These chicks were divided into four experimental groups with three replicates (12 replicates in all) each. Every replicate contained 120 birds. Every group was kept in a separate pen. These pens were demarcated by a wire mesh and the floor was covered with deep litre, using 2 inch thick wheat straw. The dimension of these pens was 6×4×4 foot. All these things were disinfected before use. After this time, the temperature of the room was kept constant at 23°C. The quail chicks were given rations *ad-libitum*. Four rations were used in all. The composition of the rations, have been given in Table 1. The proximate composition of the mustard seed meal is given in Table 2. The feeders were filled with feed twice a day and fresh water supply was made available to the chicks all the time. The water was added "VITASOL SUPER", a vitamin premix containing Vitamins A, D, E, B6, K3, B1, B2, B12, C, folic acid, niacin and calcium pantothenate. For the duration of the experiment, the photoperiod was kept at 24-h light.

Formulation of feeds

Four rations (Tables 1 and 2) were formulated and designated as A (controls), B (5% mustard seed meal), C (15% mustard seed meal), D (25% mustard seed meal) respectively. All the rations were isocaloricand isonitrogenous.

Proximate analyses

This is given in table

Weighing

The birds were weighed on the zero day (the day of hatching) and then every day till the end of the experiment to the nearest gram in order to observe the growth pattern. Every group of quails were provided the food *ad-libitum* twice a day and the left over was experiment, all the mortalities and the cause of the death were ascertained by autopsy done on the dead samples.

Sampling

As reported above, the birds were weighed at appropriate time till the age of 30 days. During this time, the samples were taken from each of the four groups from at least 5 male quails for biochemical analyses. In taking the samples care was taken to select those birds which do not deviate from the mean of the sample more than10% in weight. At the time of taking samples, the quails were slightly anaesthetized and then slaughtered. After all the blood was drained off, the liver was immediately dissected out, cleaned and blotted with tissue paper and weighed to the nearest milligram and were frozen immediately for analysis.

Table 1. Composition of different rations containing rapeseed meal fed to the Japanese quail *ad-libitum*.

Rations	Percentage rapeseed meal			
	Control	5	15	25
Maize	15	15	15	15
Wheat	25	22	20	18
Rice	11	11	9	7
Rice Polish	10	10	10	10
Sesame meal	8	8	6	2
Maize gluten	10	8	6	4
Blood meal	4	4	2	2
Fish meal	12	12	12	12
Molasses	3	3	3	3
Bone meal	0.5	0.5	0.5	0.5
Lime stone	1	1	1	1
Pre-mix	0.5	0.5	0.5	0.5
Mustard meal	0	5	15	25
	100	100	100	100

Table 2. Proximate composition of rapeseed meal used in present investigation. The meal was used after extraction of the oil.

Composition	Percent of the meal
Protein*	40
Fat**	15
Fiber	12
Ash	7.96
Moisture	6.90
Carbohydrates (Nitrogen free Extract).	18.00

*Nitrogen x 6.25, **Soxlet extraction.

Data analyses

Tissue-body index

Tissue-Body Index was calculated using the formula:

$$\text{Tissue-Body Index} = \frac{\text{Weight of organ (grams)}}{\text{Weight of animal}} \times 100$$

Statistical procedures

All weights and other values were analysed for statistical difference from respective control values by applying single-factor analysis of variance according to Sokal and Rohlf[9]. The detailed analyses were made according to Campbell [10].

Preparation of Tissue Homogenates:

A suitable amount of tissue (about 200 mg) was taken and homogenized in 1 ml ice-cold water using a motor driven homogenizer [Ultra Turrax]. Finally this homogenate was separated to measure the activity of theseLiver phosphatase enzymes; acid phosphatase and alkaline phosphatase.

Alkaline Phosphatase (Orthophophoric Monoester Phosphohydrolase)

Alkaline Phosphatase was determined by the method described by King and King [11].

Reagents

a) Buffer

6.36 grams of anhydrous sodium carbonate and 3.36 grams of sodium bicarbonate were dissolved in distilled water. pH was adjusted to 10 and volume made upto 1 litre.

b) Substrate

0.01 M disodium-phenyl-phosphate was prepared by dissolving 2.18 grams in 1 litre of distilled water, boiled, cooled quickly and preserved with 4 ml of chloroform.

c) 4-Amino-Antipyrine

6 grams of 4-Amino-Antipyrine were dissolved in one litre water.

d) Potassium Ferricyanide

24 grams of Potassium Ferricyanide were dissolved in one litre of distilled water.

e) Sodium Hydroxide (0.5 N)

20 grams of Sodium Hydroxide were dissolved in one litre of distilled water.

f) Sodium Bicarbonate (0.5 N)

45 grams of sodium bicarbonate were dissolved in one litre distilled water.

g) Stock Phenol Standard

One gram of pure crystalline phenol was dissolved in one litre of 0.1N HCl.

h) Working Phenol Standard (1 mg/100 ml)

1 ml of stock solution was diluted upto 100 ml with distilled water.

Procedure

For the sample tubes I ml of buffer (pH 10) with 1ml of phenyl phosphate were taken in a tube and placed in a water bath at 40±1^{o}C for 3 minutes. Then 0.1 ml of tissue homogenate were added and mixed. Tubes were incubated for exactly 15 minutes. The reaction was then stopped by adding 0.8 ml of 0.5N sodium hydroxide. Then 1.2 ml of 0.5N sodium bicarbonate, 1 ml of amino-antipyrine and 1 ml of potassium ferricyanide were added in the above order. Tubes were mixed after each addition and colour formed was read against a reagent blank at 510 nm. Known concentration of phenol solution was used as standard. The activity is expressed as moles of phenol released/min/100 mg of tissue.

Acid Phosphatase (Orthophophoric Monoester Phosphohydrolase)

Acid phosphatase was determined by the King and Jegatheesan [12] method. Same reagents as in determination of alkaline phosphatase except that citrate buffer was prepared by dissolving 42 grams of citric acid in 200 ml water, 376 ml of 1N sodium hydroxide was added and the volume made upto 1 litre with water and pH was fixed at 4.9.

Procedure

One ml of citrate buffer (pH 4.9) and 1 ml of substrate were incubated at 40±1oC for 3 minutes. Then 0.2 ml of tissue homogenate was added to each tube and incubated for exactly one hour. Reaction was stopped by the addition of 1 ml of 0.5N sodium hydroxide. The 1 ml of 0.5N sodium bicarbonate followed by 1 ml of amino-antipyrine and 1 ml of potassium ferricyanide solution. Tubes were shaken well after each addition and colour formed was read against a reagent blank at 510 nm. Standard phenol solution was used for calculations. The values are described as given for alkaline phosphatase.

Statistical Procedures:

All values were analyzed for statistical difference from respective control values by applying Single-Factor Analysis of Variance according to Sokal and Rohlf [9]. The detailed analyses were made according to Campbell [10].

RESULTS

Alkaline Phosphatase At zero-day, the Alkaline Phosphatase had theactivity in the liver to the time of 0.68 u mole of nitro-phenol formed/min/100mg. After decreasing the values between 8-10 days,the activity of enzyme increased till the end of the experiment. At 14 days there was no significant difference between the groups but at the end of the experiment a significant activity difference was seen among the different groups (ANOVA; P<0. 001) (Table 3,4,5).

Acid Phosphatase At zero-day this lysosomal enzyme had values 13 times less than the Alkaline Phosphatase. The actual values were 0.05±0.002 u mole of nitro-phenol formed/min/100mg of the liver. The values decreased upto 8 days and then these values started increasing till 22 days of life. After this time the values decreased again. At 14 days 15% had higher values than other groups (P<0.01). At 30 days, 15% and25% had higher (P<0.05) values than the controls (Table 6, 7, 8).

Table 3.Effect of feeding different percentages of Rapeseed meal on the Alkaline Phophotase (u Mole Nitrophenol formed/min/100mg) activity of Liver of Japanese quail. Values given are Mean±S.E. of 5 animals each. For statistical analysis, see tables 4 and 5.

Age (days)		Percentage rapeseed meal in diet		
	Control	5.0	15.0	25.0
Zero day	0.68±0.04	-	-	-
6	0.26±0.02	0.33±0.02	0.30±0.01	-
8	0.29±0.03	0.31±0.03	0.22±0.02	0.24±0.02
10	0.63±0.12	0.42±0.05	0.20±0.02	0.20±0.002
14	1.46±0.16	1.80±0.21	1.55±0.11	1.22±0.18
18	1.87±0.16	1.52±0.29	2.13±0.22	1.68±0.11
22	2.63±0.02	2.42±0.06	3.24±0.32	2.59±0.10
30	2.76±0.33	1.88±0.25	3.66±0.23	3.18±0.14

Table 4.Detailed statistical analyses based on the given in table 1. Statistics according to single factor analysis of variance.

ALKALINE PHOSPHOTASE(14 DAYS)

ANOVA TABLE

Items	**Sum of squares**	**Df (n-1)**	**Mean squares**	**F-value**
Rations	0.851775	3	0.283925	1.6153441 (not significant)
Error	2.81228	16	0.1757675	
Total	3.664055	19		

Table 5.Detailed statistical analyses based on the given in table 1. Statistics according to single factor analysis of variance.

ALKALINE PHOSPHOTASE (30 DAYS)

ANOVA TABLE

Items	**Sum of squares**	**Df (n-1)**	**Mean squares**	**F-value**
Rations	6.83602	3	2.2786733	6.9234282(P<0.01)
Error	3.95175	12	0.3293125	
Total	10.78777	15		

DETAILED COMPARISONS BETWEEN RATIONS

Comparison			F-Value	Significance
Control	Vs	5%	4.6764471	P<0.05
Control	Vs	15%	4.9193395	P<0.05
Control	Vs	25%	1.0841146	N.S
5%	Vs	15%	19.188499	P<0.001
5%	Vs	25%	10.263807	P<0.01
15%	Vs	25%	1.38474309	N.S

Table 6. Effect of feeding different percentages of Rapeseed meal on the Acid Phophotase (u Mole Nitrophenol formed/min/100mg) activity of Liver of Japanese Quail. Values given are Mean±S.E. of 5 animals each. For statistical analysis, see tables 7 and 8.

Age (days)		Percentage rapeseed meal in diet		
	Control	5.0	15.0	25.0
Zero day	0.05±0.002	-	-	-
6	0.04±0.003	0.02±0.0008	0.01±0.003	-
8	0.02±0.002	0.01±0.002	0.02±0.0005	0.01±0.001
10	0.05±0.01	0.03±0.002	0.12±0.003	0.11±0.003
14	0.10±0.004	0.08±0.002	0.12±0.003	0.11±0.003
18	0.27±0.01	0.21±0.01	0.26±0.01	0.22±0.008
22	0.36±0.03	0.31±0.03	0.36±0.03	0.36±0.01
30	0.19±0.008	0.21±0.02	0.28±0.02	0.27±0.03

Table 7. Detailed statistical analyses based on the given in table 4. Statistics according to single factor analysis of variance.

ACID PHOSPHOTASE (14 DAYS)

ANOVA TABLE

Items	**Sum of squares**	**Df (n-1)**	**Mean squares**	**F-value**
Rations	0.00428	3	0.00142666	5.4615385
Error	0.00372	14	0.000265714	
Total	0.008	17		

DETAILED COMPARISONS BETWEEN RATIONS

Comparison			F-Value	Significance
Control	Vs	5%	0.000680555	Not Significant
Control	Vs	15%	5.419462	P<0.05
Control	Vs	25%	1.2043658	N.S
5%	Vs	15%	39.565299	P<0.001
5%	Vs	25%	6.7745578	P<0.05
15%	Vs	25%	1.2043658	N.S

Table 8. Detailed statistical analyses based on the given in table 4. Statistics according to single factor analysis of variance.

ACID PHOSPHOTASE (30 DAYS)

ANOVA TABLE

Items	**Sum of squares**	**Df (n-1)**	**Mean squares**	**F-value**
Rations	0.0300409	3	0.0100136	3.7436818 (P<0.05)
Error	0.0427976	16	0.00267485	
Total	0.0728385	19		

DETAILED COMPARISONS BETWEEN RATIONS

Comparison			F-Value	Significance
Control	Vs	5%	0.6221399	Not Significant
Control	Vs	15%	7.4033573	P<0.05
Control	Vs	25%	6.8805518	P<0.05
5%	Vs	15%	3.7332137	N.S
5%	Vs	25%	3.367376	N.S
15%	Vs	25%	0.0095708	N.S

In this study, Biochemical studies were performed on the liver show that at 30 days age the activity of the acid phosphatase decreased but the activity of alkaline phosphatase did not, which had been reported in the research for mammals and other domestic birds.

DISCUSSION

The present study was undertaken to observe the effects of feeding *Brassica* seed meal on the Nucleic acids of Japanese quail *Coturnix coturnix japonica.*As reported earlier seeds belonging to the family Cruciferae contain certain toxic substances, which upon inclusion in the diet cause growth retardation, thyroid hypertrophy, liver damage and other biochemical and metabolic derailment. The Brassica seeds were purchased from the local grain market and were expelled from an oil expeller to extract oil from them. This meal obtained after removing the oil from the seeds was incorporated in isocaloric and iso nitrogenous diets at the rate of zero, 5, 10, 15, 20 and 25% of the diet [13]. The diets were prepared from the local ingredients and were designed to be low cost diets. The feeding of these diets was started from the day of hatching and continued upto 4-weeks of age.

A set of two enzymes, i.e., alkaline phosphatase and acid phosphatase were measured in the hepatic tissue. The pattern of development of these enzymes was quite similar to each other. The values being quite low for acid phophatase; an enzyme used also as an index of lysosomal activity. The value of the enzyme decreased in the first 10 days of age. This decrease being more marked in alkaline phosphatase. After this time, the enzyme started increasing and after 22 days, their behavior differed again. The activity of the acid phosphatase decreased whereas, the activity of alkaline phosphatase did not. There was also statistical difference between the experimental and controls at 30 days in both enzyme activities. The changes reported herein were seemed to have more of the manifestation of the age rather than the effect of glucosinolates. Studies were performed to show that glucosinolates present in the mustard did not have the drastic effects which have been reported in the literature for mammals and other domestic birds

The present results are nearly four times the figure obtained by [14] in control positive and negative control groups of Japanese quails. The present findings are in consonance with the results obtained by Iqbal[15] in fowls, the primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. When the liver, bile ducts or gallbladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the blood stream leading an increase in the values. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine [16].

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